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Entering the Terra Incognita between Biochemistry and Cytology

Putting New Research Tools to Work in the 1940s

> In recent years, the construction of a bridge between these two levels of knowledge has been initiated. This has been due to the adaptation and employment in biology of techniques derived from physics and chemistry and to the breaking down of the barriers which previously separated these sciences. Below the structure visible to the microscope there exists a true organization of molecules and micelles in the different phases of the system which constitute protoplasm.
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> (de Robertis et al., 1949, p. 64).

New research tools, especially cell fractionation and electron microscopy, opened for investigation the uncharted territory between biochemistry and cytology. The goal was to explain how cells carry out their basic functions. In the nineteenth and early twentieth century, the activity that had received the most attention was cell reproduction. The basic operations in cell division, including those carried out by chromosomes in the nucleus, had been described by cytologists using stains and the apochromatic lens decades before the advent of cell biology. The focus of early cell biology was rather on functions performed in the cytoplasm, especially capturing energy and synthesizing proteins. The first important steps in developing mechanistic explanations of these functions was to identify the mitochondrion and the endoplasmic reticulum as the cell organelles responsible for each, a project largely accomplished in the 1940s (although the name *endoplasmic reticulum* was not introduced until the early 1950s).

One laboratory at the Rockefeller Institute, in which Albert Claude, Keith Porter, George Palade, and others performed pioneering research, played the pivotal role in establishing these structure–function linkages. As we will see, understanding cell function was not the initial objective of research in this laboratory, and it was only at the end of the 1940s that it officially became

a laboratory for cell biology. Hence, it is important to understand the transformation in this laboratory. Other laboratories, ones initially more explicitly devoted to cell physiology, also made significant contributions. At appropriate points in the chapter I will briefly profile three of them. It was the laboratory at the Rockefeller Institute, though, that established the model for what cell biology was and how it was done, and that will be the main focus of this chapter.

1. FIRST STEPS TOWARD CELL BIOLOGY AT THE ROCKEFELLER INSTITUTE: CLAUDE'S INTRODUCTION OF CELL FRACTIONATION

The pioneering investigations in cell biology at the Rockefeller Institute occurred in the cancer laboratory of James Murphy. Murphy was pursuing a line of research that traced back to Peyton Rous, whom Simon Flexner, the first director, recruited to the Rockefeller Institute in 1909. Shortly after arriving at Rockefeller, Rous was presented with a chicken with a large lump on its leg. The lump turned out to be a tumor that Rous showed could be transmitted from one animal to another by inoculation either with small portions of the tumor or with a filtered extract that strained out all cancer cells. He concluded that the tumor was carried by what were then termed "viral agents," characterized only as infectious entities that were not bacterial in nature. Although the tumor was to bear his name, Rous had been a reluctant recruit to cancer research and in 1915 turned instead to research on blood preservation (Corner, 1964).

With the change in direction of Rous' research, Murphy, who had been Rous' assistant, was promoted to associate member of the Institute and placed in charge of cancer research. (He became a full member in 1923.) For a number of years, his primary focus was on the possible role of lymphocytes in resisting cancer (an inquiry rooted in an observation he and Rous had made that embryos and brain tissue, both lacking lymphocytes, lacked resistance to transplanted tumor cells). By the late 1920s, though, Murphy turned his attention back to the agent responsible for Rous chicken sarcoma. Rous had shown not only that the suspected causal agent was found in cell-free extract but also that tumor cells could be killed with ultraviolet light without destroying the agent, which suggested to Murphy that the Rous chicken tumor agent might not be bacterial but rather have an "enzyme-like nature" (Murphy, Helmer, & Sturm, 1928). He had also determined while working under Rous that freezing and drying the tissue (a process known as lyophilization) did

not kill the active agent. He now sought to purify this agent and turned that task[1] over to a new assistant, Albert Claude, who had received his M.D. at Liége in 1928 and spent a year in a tissue-culture laboratory in Berlin before Murphy recruited him in 1929.

Claude pursued a number of strategies in the attempt to purify the agent, including adsorption, precipitation, and dialysis. Precipitation of carbohydrate with gelatin followed by dialysis resulted in a twentyfold "enrichment of the tumor producing agent" (Claude, 1935). He identified a protein and a phospholipid as the principal constituents of the active residue. None of these techniques, however, produced the desired purification.

At this point Claude read the report of two British researchers, Ledingham and Gye (1935), who had tried using high-speed centrifugation to separate a tumor producing agent (also McIntosch, 1935, who employed a Henriot and Huguenard air-driven ultracentrifuge). Although the substance Ledingham and Gye isolated had less tumor producing capacity than the initial cell extract, Claude saw the promise of their approach and set out to refine it. In 1937 he first reported on using a high-speed centrifuge to isolate a more potent tumor causing agent (Claude, 1937)[2] and in 1938 he claimed even better results – a tumor-causing agent with ten to fifty times greater potency than the original extract (Claude, 1938b). In the 1938 *Annual Report* Claude began to characterize the chemical makeup of the active particle, arguing that it contained phospholipids as well as ribosenucleic acid. The chemical composition of the tumor-producing fraction was also the focus of a further paper (Claude, 1939). Viewing the fraction under a dark-field microscope, he observed it to consist of small granules approximately 70 mμ in diameter.

As a control to his work with tumor cells, Claude (1938a) centrifuged normal tissues, and discovered to his surprise a fraction with granules similar to that generated by centrifugation of tumor cells. Its chemical composition was also similar: "The chick embryo material, like the tumor fraction, is

[1] Murphy continued to work on tumors of fowl with Sturm and was the official head of the laboratory until his retirement in 1949. He focused on such topics as chemical induction of tumors (by dibenzanthracene, for example) and their transmissibility. He also examined the effect of such variables as season of year and genetic constitution on susceptibility to the cancers.

[2] In the 1937 *Annual Report*, Claude described introducing centrifugation to address the problem of why a related tumor, Chicken Tumor 10, could be transferred through filtrates. In 1931 Murphy and Claude had shown that alumina gel had to be added to produce an active filtrate with Rous sarcoma. They proposed that the alumina gel removed an inhibitor. Claude now argued that the problem with Chicken Tumor 10 was also due to an inhibitor by showing that the centrifuged particle when combined with water could generate the tumor. When the particle was combined with the supernatant from the centrifugation it could not do so, suggesting that the supernatant contained an inhibitor.

found to consist essentially of a phospholipid-aldehyde portion, associated with a nucleoprotein of the ribose type" (Claude, 1939, p. 214). He concluded, "These observations indicate that a phospholipid-ribose nucleoprotein complex is probably a general constituent of normal and tumor cells" (p. 215). This surprising result led Claude to shift his research away from the cancer paradigm and toward an investigation of the constituents of normal cells.[3]

In the following year Claude began to consider the hypothesis (later found to be false) that these granules might be mitochondria and advanced several bits of evidence in support: they (a) were of similar size and shape as mitochondria (based on size estimates by Cowdry, 1918), (b) had a similar composition of phospholipids and nucleoproteins, and (c) exhibited similar responses to heat and acids (Claude, 1940; Claude, 1941). In the 1940–1 *Annual Report*, he advanced an additional piece of evidence: "Janus green has a selective affinity for mitochondria under proper conditions and the isolated particles show an identical property. With methyl green the cell nucleus takes a brilliant green while the mitochondria become purple. The isolated particles take the same purple color with this dye" (p. 72).

Although Claude initially misidentified the granular particles as mitochondria, he later corrected himself and made landmark contributions to the understanding of both the granular particles and mitochondria. The misidentification does not detract from the general point that, having recognized that some of his findings with tumor cells pointed to important components of cells more generally, Claude's research program was radically transformed. This involved not simply turning in a different direction, but as Rheinberger (1995) has emphasized, arriving at a quite different conceptual framework as he reinterpreted his work: "What had been the tumor agent, 'was' now a cytoplasmic particle. The new scientific object arose and began to be delineated first as an intrusion, then as a supplement within the confines of the old cancer research system. Shortly after, it took over and transformed the system itself" (p. 61).

[3] The shift, however, was not total. In a talk at the American Association for Cancer Research on 19 April 1941, Claude offered a proposal as to how putative mitochondrial particles related to cancer. From the fact that what he took to be mitochondria seem to increase in number in cells when they are dividing, he inferred that they figure in the process of cell differentiation. He then proposed, "The nature of the cellular response to cancer-inducing chemicals suggests that the system which is affected is probably that part of the cell which normally influences differentiation and growth" (*New York Times*, 20 April 1941). Claude went on to note that other researchers had linked mitochondria to cell respiration, and adduced evidence that some cancer causing agents also interfere with respiratory enzymes.

2. ROBERT BENSLEY: AN ALTERNATIVE APPROACH TO FRACTIONATION

In changing his focus to normal cells, Claude drew close to the work of a more senior investigator, Robert Bensley of the University of Chicago (apparently Claude was initially unaware of Bensley's work). Just a couple of years earlier, Bensley had arrived at – and applied – the idea of using centrifugation as a means of separating mitochondria from cells. Bensley was a traditional cytologist whose career spanned the first four decades of the twentieth century. He started to explore cell staining techniques while recovering from a hunting accident in his early teens that cost him one of his legs. Already in the first decade of the twentieth century, Bensley was conducting research on mitochondria and the Golgi apparatus. In particular, he further developed procedures for fixing cells with acetic-osmic-dichromate and staining them with anilin-acid fuchsin and methyl green and copper-chrome-hematoxylin that proved useful for targeting mitochondria. Bensley stands out from the other major cytologists active in the first decades of the twentieth century, though, in that late in his career he pioneered a means of bridging from morphology to chemistry. This was necessary if the structural decomposition of the cell offered by cytologists was ever to be linked to a functional decomposition (especially to the level of biochemical reactions). Whereas we saw in Chapter 3 that his former student Cowdry abandoned research on mitochondria in the 1930s, having become pessimistic about the prospects for finding such a bridge, Bensley pursued his idea with two of his last graduate students, Isidore Gersh and Normand Hoerr.

As discussed in the previous chapter, Gersh (1932) revived and simplified Altmann's procedure for fixing cells without chemical reagents by freeze-drying. Bensley and Gersh (1933b) used the technique to test many older claims about the effects of different solvents and heat on mitochondria. Many of the fat solvents, such as acetone, had no effect on mitochondria. On the other hand, water, 0.02% ammonia solution, and artificial gastric juice dissolved them. Bensley and Gersh interpreted this as showing that "the main mass of the mitochondria substance is protein in nature" (p. 230). Bensley together with Hoerr then took the major step of employing centrifugation to isolate mitochondria from fresh liver tissue preparations. They prepared specimens by perfusing the liver with salt solution, then grinding it in a mincing machine and pushing the result through a sieve of cheesecloth.[4] Several successive

[4] This paper, which reported results that did not rely on the freeze-drying method, appeared as the sixth in a series entitled "Studies on cell structure by the freezing-drying method." The connection

centrifugations at slow speed then removed nuclei, blood corpuscles, and connective tissues. They then centrifuged the remaining supernatant at higher speeds for longer periods, which caused mitochondria to sediment. Bensley and Hoerr's goal was to analyze their chemical constitution. Although they expressed doubt in their first paper about the fat composition of mitochondria (Bensley & Hoerr, 1934a), their second paper showed mitochondria to be comprised of, on average, 43.6% fat by weight, but not to contain lecithin or cephalin (Bensley & Hoerr, 1934b). They also showed that precipitation at different acidities revealed the presence of least two proteins.

In further research, Hoerr (1943) refined the techniques for isolating cell components and Bensley (1937) refined the analysis of the constitution of mitochondria. Arnold Lazarow, another of Bensley's students, continued the chemical analysis of the constitution of mitochondria and also discovered a smaller particle that appeared cherry red when separated by centrifugation. He produced quantitative analyses of both the original and smaller particles, showing that the smaller particle contained more phosphorus and fat than did the mitochondrial particles. He demonstrated that both the mitochondrial fraction and the smaller particle (which he referred to as *the submicroscopic lipoprotein component*) oxidized succinic acid, which he interpreted as showing that both contained succinic dehydrogenase, cytochrome *c*, and cytochrome oxidase. Lazarow's smaller particles were in fact the ones Claude was finding at the same time and misidentifying as mitochondria. He also investigated their enzyme constitution several years before that became a focus in Claude's laboratory at the Rockefeller Institute. With regard to the submicroscopic lipoprotein component, Bensley commented on their common timing:

> I had no suspicion at first that still smaller particulates were present in the liver cell until Lazarow, by long-continued centrifugation, obtained a glassy cherry-red pellet composed of particles so minute that they were quite invisible under the microscope, but showed in the dark-field of the cardioid condenser a shimmering field of light in which individual particles could with

is found in the previous paper, also coauthored by Bensley and Hoerr, in which emulsions of fresh liver cells prepared by three different methods – grinding in a mortar, mincing in a latapie grinding machine, or kneading through bolting silk – were used to provide a check on results from freeze-drying. Their focus in that paper was on contents that remained once the nucleus and (putative) mitochondria were removed. They argued for the existence of a protein structure they dubbed "elipsin" which "by itself maintains the cell as a unit of organic structure after the soluble globulins, mitochondria, and chromatin have been removed seriatim by solution [and] is in reality the basis of the microscopic structure and of the organic continuity of the cell body" (Bensley & Hoerr, 1934a, p. 263).

> difficulty be distinguished. We were investigating this particle when Claude (1940) announced his discovery of the presence of submicroscopic particulates in clarified saline extracts of embryo chicks. (1943, p. 329)

Bensley and his graduate students pioneered many of the advances for which Claude and the Rockefeller group were to get most of the credit. In 1943, Cowdry referred to Bensley as "the acknowledged founder of the new cytology" and adds, "As the lesser figures shrink and are forgotten his stature will grow" (1943, p. 8). Cowdry's prophecy, however, turned out to be erroneous as Claude and the Rockefeller group rapidly eclipsed Bensley.[5] One reason might well be Bensley's advanced age when he was pursuing this research – he was already professor emeritus. Another, perhaps not trivial, factor is that Bensley did not think of mitochondria as permanent structures but as coacervates that appear and then are reabsorbed into the protoplasm (a view Bensley held until the end of his career, see Bensley, 1953). The most important factor that made Claude and his laboratory the crucial locus for developing cell biology is that they – not Bensley – convincingly established the role of the mitochondrion in cellular energetics, thereby opening the productive endeavor of linking function, determined biochemically, to structure, identified cytologically.

3. COMPETING INTERPRETATIONS OF FRACTIONS FROM NORMAL CELLS

Although both Bensley's group and Claude differentiated two fractions, their initial interpretations of how these related to cell structures conflicted. In appealing to Cowdry (1918) for information about mitochondrial size in arguing that his small particles were mitochondria, Claude ignored Bensley and Hoerr's more recent estimates. According to Bensley and Hoerr, mitochondria were considerably larger than Claude's small particles.

Claude (1941) discussed the differences between his fractions and Bensley's at length. He devised a way of separating two fractions through successive centrifugation runs. One consisted of smaller particles (the size of his original preparation) and the other of larger particles (the size Bensley had identified as mitochondria). Having distinguished them, Claude maintained

[5] The American Association of Anatomists named an award for Bensley, which is described in the following terms: "The R. R. Bensley Award recognizes 'rising stars' in cell biology, who have already advanced anatomy through the study of cell biology. The award is presented to someone who has made a distinguished contribution to the advancement of anatomy through discovery, ingenuity, and publications in the field of cell biology."

that the smaller particles were mitochondria and argued that those in what he called the "large granule fraction," which corresponded to Bensley's particles, were secretory. Claude's analysis of the chemical make up of the smaller and larger particles indicated only minor differences (for example, both seemed to contain phospholipids, although the smaller particles contained about twice the percentage, and contained ribose nucleic acids, iron, and copper). Claude tentatively adopted a proposal put forward by Noel in 1923 that the large particles have their "origin in a progressive transformation of the smaller elements, or mitochondria" (p. 269).

In the discussion following the paper, Jack Schultz raised the possible relevance of another constituent of the cell proposed in the era of light microscopy, the ergastoplasm. As we will see, Schultz had collaborated with Caspersson at the Karolinska Institute in the 1930s, where they had proposed and advanced evidence that RNA played a role in protein synthesis. In a very few years, most researchers would view the small particles, ergastoplasm, and RNA as interrelated, but Claude held instead that the ergastoplasm did not exist: "In recent years, the majority of workers have come to consider the ergastoplasm as an artefact produced, as a rule by fixatives containing strong acids. It is rare to find well preserved mitochondria and ergastoplasm simultaneously in the same preparation and the suggestion has been made that the ergastoplasm was merely poorly fixed mitochondria" (p. 270). In support of this position, he cited a review by Bowen (1929).

In the period that followed, however, Claude came to recognize that his small particles were too small to be mitochondria, which themselves were large enough to be visible in the light microscope. He also tried traditional stains on the particles in the small fraction and discovered they stained differently than mitochondria. As a result, he radically altered his interpretation, construing the small particles now as a newly discovered cell constituent:

> the evidence, so far, indicates that the mass of the small particles does not derive from the grossly visible elements of the cell but constitutes a hitherto unrecognized particulate component of protoplasm, more or less evenly distributed in the fundamental substance and which imparts to it, in well-preserved preparations, its staining properties. In order to differentiate the small particles from the other, already identified elements of the cell, it may be convenient in the future to refer to this new component under a descriptive name which would be specific. For this purpose the term *microsome* appears to be the most appropriate. (Claude, 1943b, p. 453)

Claude now needed to place mitochondria elsewhere, so he reinterpreted his "large granule" fraction as most likely an impure mixture that included

mitochondria. Unlike Bensley and others, though, he continued to maintain for years that secretory particles were a major component as well:

> Secretory particles are abundant in the guinea pig liver, especially in the fasting animal, where they accumulate and seem to fill the cell completely, and it appears probable that up to the present, mitochondria have not been isolated in a pure or concentrated form, a large part of the so-called "mitochondria" fraction representing probably, to a large extent, mature secretory granules. (Claude, 1943b, p. 455)

In 1943 Claude also began to employ a different procedure, centrifuging whole liver cells from *Amphiuma tridactylum* for one hour at 18,000 *g*, causing the cell contents to separate into distinguishable layers while remaining within the cell membrane. By staining the cells with Bensley's stain (acid fuchsin-methyl green), he rendered what he took to be secretory granules and mitochondria vivid red against a purple background (Claude, 1943a). This yielded a sharp distinction between four successive layers, which Claude interpreted as (1) glycogen, (2) a combination of secretory granules and mitochondria that appeared red and also contained nuclei, (3) the "purple substance" that he took to be microsomes, and (4) the true hyloplasm (cytosol).

In general, the Bensley group welcomed Claude's entry into the field of centrifugation studies that they had pioneered. He was an invited speaker at the symposium in November 1942 in honor of Bensley's seventy-fifth birthday, and many of Bensley's colleagues referred positively to his contributions (Hoerr called his work "superb"). However, they challenged his refusal to identify his large-granule fraction as essentially mitochondrial and his insistence that many of the granules were secretory. Hoerr said, "it is obvious that he has separated the liver mitochondria" (1943), appealing to the ability of staining to differentiate between mitochondria and secretory granules when they are present in, for example, pancreas cells. He further criticized Claude's methods of separation, arguing that they failed to produce pure preparations but rather yielded mixtures. Just as Claude continued to refer to *large granules* in preference to *mitochondria*, so Bensley's group rejected Claude's term *microsomes*, instead referring to the small particles as *submicroscopic particulates*.

The following year Claude (1944) offered yet another reason for questioning whether the large granules extracted from guinea pig liver included mitochondria – they failed to stain with Janus green. Now he concluded that liver may be a poor place to look for mitochondria, and reported results of a study on leukemic cells from rats, in which he contended that the large granule fraction consisted of true mitochondria. He then generated a chemical

analysis very similar to that which he had offered for the large granules that he took to be secretory in liver. Over the next several years, he persisted in referring to the *large granule* fraction, acknowledging that mitochondria were part of the fraction, but continuing to focus on the idea that it also contained secretory granules.

4. LINKING CLAUDE'S MICROSOMES TO PROTEIN SYNTHESIS

Claude's analysis of his "hitherto unrecognized particulate component of protoplasm" – what he called microsomes – indicated the presence of RNA. Although Claude was hesitant to suggest a function for these particles, two other investigators, Jean Brachet and Torbörn Caspersson, led the way. Each deployed new cytochemical tools to establish the connection between RNA and protein synthesis.

Brachet: Selective Staining of RNA and Correlation with Protein Synthesis

While still an undergraduate medical student at the Free University of Brussels in the late 1920s, Brachet tried to determine the location of what was then referred to as thymonucleic acid[6] (DNA) in growing oocytes. At the time, thymonucleic acid was thought to occur only in animals while zymonucleic acid (RNA) was found primarily in plants (although it was also recognized to be present in the pancreas in animals). The discovery of the Feulgen reaction (Feulgen & Rossenbeck, 1924), which stains DNA green, made it possible for Brachet to try to localize DNA, but it was zymonucleic acid or RNA that was to be the focus of his major breakthrough. Needham and Needham (1930) had found large amounts of nucleic acids in sea urchin eggs, which stained intensely red with pyronine. Was this RNA? Brachet found that when he treated the stained cells with ribonuclease, which specifically digests RNA, this removed the red stain, indicating that it was RNA. Brachet hypothesized, based on the fact that the amount of RNA was closely correlated with protein synthesis activity, that RNA played a role in protein synthesis: "The conclusion to which we are led is that the pentosenucleic acids [RNA] might intervene, by a mechanism as yet obscure, in the synthesis of proteins, which

[6] The discovery that the substance contained deoxyribose, which led to its rechristening as *deoxyribose nucleic acid* (DNA), only came with the research of Phoebus Levene and Takajiro Mori (1929) at the Rockefeller Institute.

perfectly matches the available facts" (Brachet, 1942, p. 239, as quoted in Rasmussen, p. 60).

As he was completing the ribonuclease research, Brachet and his student Hubert Chantrenne began a collaboration with biochemist Raymond Jeener in which they isolated particles similar to those Claude had identified as microsomes using a prototype of Emile Henriot's air-driven centrifuge. They called these "cytoplasmic particles of macromolecular dimension" (Brachet & Jeener, 1944) and claimed that nearly all cytoplasmic RNA was located in them, together with several hydrolytic or respiratory enzymes. They also speculated that the hydrolytic enzymes could be caused to work in reverse through the energy released by the respiratory enzymes in order to synthesize peptide bonds.

World War II had a substantial impact on Brachet and his research program. The Germans, occupying Belgium, closed Brussels University in 1942 and Brachet was arrested and imprisoned for nearly three months.[7] He briefly resumed work in Liége until Allied bombing made this impossible. During this hiatus from research, he wrote an overview of chemical processes in development entitled *Chemical Embryology*. After the war, Brachet reestablished his laboratory, although under difficult financial circumstances,[8] in small houses at the university's botanical gardens on the outskirts of Brussels. Relatively quickly, he reassembled his network of collaborators. Chantrenne was appointed professor of biochemistry and he and Brachet continued to investigate RNA metabolism during protein synthesis, the effects

[7] Brachet describes the period: "The interdiction, made by the Germans in 1942, to all members of the staff to enter any more the University laboratories obliged us to stop that sort of work; a difficult, but successful, task was the hiding of all the laboratory equipment, including the instruments given by the Rockefeller Foundation in 1938. A search was made for some of these American instruments by the Germans, but they were not to be found. They are of course in constant use in the laboratory now. My arrestation as an hostage and emprisonment in a fortress for 2½ months, followed by a 3 months necessary rest after my release, meant a long interruption of my work in 1943" (Report of activity since 1940, folder 38, box 4, Series 707D, RG 1.2, Rockefeller Foundation Archives, RAC).

[8] Brachet at this time seriously considered emigrating, but concluded that the prospects for improved conditions were sufficient not to pursue that option. He reported in a summary to the Rockefeller Foundation, "As a matter of fact, Brussels University has recently been backing me as far as it can: a new technician has just been appointed in the laboratory and money (10,000 frs) has been given with the purpose of getting a larger ultracentrifuge in working order; it is likely that ultracentrifuge studies will be resumed in a few weeks. The University is also intending to build new laboratories, where I shall find my place among chemists and physicists; the Board of Trustees has also accepted my proposal of the creation of the 'groupment d'études de biologie physicochique', which will help in tightening the links between biologists, chemists and physicists" (Report of activity since 1940, folder 38, box 4, Series 707D RG 1.2, Rockefeller Foundation Archives, RAC).

of ribonuclease on living cells, and the role of the nucleus in the synthesis of RNA and proteins. Jeener became professor of animal and comparative physiology. Although space was very limited, a substantial number of foreign visitors spent periods working in the various laboratories. In the late 1950s, new buildings were finally constructed for Chantrenne, which included an electron microscope purchased with funds from the Rockefeller Foundation.

In a letter to Pomerat on 27 May 1961, describing the dedication of the new electron microscope facility, Brachet also noted that he was "fighting on other grounds (money coming from the Government) for the support of Cell Biology and the development of Molecular Biology in Belgium."[9] As Burian (1996) discussed, the research by Brachet and his colleagues in the late 1940s and early 1950s on different forms of RNA and their role in protein synthesis contributed significantly to the development of molecular biology. Despite his important contribution in linking protein synthesis to RNA and RNA to the particles Claude labeled *microsomes*, Brachet's research did not exert the impact that the Rockefeller laboratory did in developing the new field of cell biology. An important factor may have been the fact that Brachet and his collaborators focused solely on RNA and protein synthesis. Another is that electron microscopy was not central to their research. Probably even more important was that even as they employed cell fractionation, they were not committed to the differentiation of distinct fractions representing different functions. It was the breadth of Claude's emerging vision that enabled the Rockefeller laboratory to set the agenda for the new discipline of cell biology.

Caspersson: Spectrographic Analysis, RNA, and Protein Synthesis

During the same period in which Brachet was linking microsomes, RNA, and protein synthesis, Caspersson was making similar connections at the Karolinska Institute in Stockholm. Caspersson had been a student of Einar Hammarsten, professor of chemistry at the Karolinska, who had himself been conducting research on protein chemistry, including relations between nucleic acids and proteins. In 1944, Caspersson was appointed to a new professorship in cell research at the Karolinska. Another Hammarsten student, Hugo Theorell, had been appointed as the first research professor of the Medical Nobel Institute in 1938, and the Nobel Institute provided funding for a new building to house both Theorell's and Caspersson's laboratories. Substantial grants from the Wallenberg Foundation and from the Rockefeller Foundation, which had been a long-time supporter of Hammarsten's research,

[9] Folder 40, Box 5, Series 707D, RG 1.2, Rockefeller Foundation Archives, RAC.

helped establish both laboratories. Thus, well before the Rockefeller group, Caspersson had a well-funded laboratory dedicated to cell research.

Caspersson tended to have more of an interest in the development of instruments than in the biological research that they made possible. When he obtained his own laboratory space, a workshop for building instruments was a major component. Discussion of refinement of instruments typically appeared ahead of experimental results in Caspersson's annual reports to the Rockefeller Foundation.[10] While working under Hammarsten, he developed methods for ultraviolet spectrography that involved combining a spectroscope and a microscope with a quartz lens. Relying on the fact that the pyrimidines in nucleic acids strongly absorb light at 2600 Å, he was able to estimate nucleic acid content in different parts of living cells. In 1938, in awarding a further grant to Hammarsten, the Rockefeller Foundation took note of this equipment: "The equipment as it now stands is the result of accretions and modifications made as new needs and possibilities were uncovered. In a certain sense the equipment is relatively crude, although it is substantial enough to indicate the limits and possibilities of this type of analysis."[11] In applying for that support, Hammarsten described Caspersson's photoelectric apparatus for ultraviolet-microspectrography (letter to W. E. Tisdale, February 23, 1938):[12]

> Having passed the living cells the light is concentrated in the microscope with an iris and a quartz-prism over the ocular. By means of fluorescent glass and mirrors it is possible to get an orientation in the light-bundle, and by movements of

[10] In a report on December 9, 1953, Caspersson described the division between instrument development and biological research: "the work in the institute has been carefully divided so that half the resources were devoted to developmental work on the side of the instruments and the other half to work on biological problems with the intracellular regulation of protein synthesis as key note. This arrangement has always very strictly been carried through, in spite of the fact that it has often been evident that the biological work on short sight would have benefited from a larger share of the efforts, that would undoubtedly also have made the work more easy to manage financially. The reason for this politics [sic] was that the biophysical techniques in question are the primary condition for the work, and furthermore they represent in my personal view one of the ways, which has to be gone sooner or later if we will ever get close to the basic problems of gene reproduction and gene function and thus a quite general approach from the beginning should prove the most fruitful at the end" (Folder: Karolinska Institutet Cell Research 1953, Series 800D, RG 2, Rockefeller Foundation Archives, RAC).

[11] Folder 5, Box 1, Series 800D, RG 1.1, Rockefeller Foundation Archives, RAC.

[12] Folder 1, Box 7, Series 800D, RG 1.1, Rockefeller Foundation, RAC. Plans were already in place for Caspersson to be a Rockefeller Foundation fellow with Lewis. These were initially postponed because Jack Schultz (then a postdoctoral fellow at the California Institute of Technology under T. H. Morgan) went to Stockholm to work with Caspersson. The outbreak of World War II then prevented Caspersson's travel to the United States; the Rockefeller Foundation provided Caspersson grants-in-aid throughout the war.

> the prism to move the enlarged picture along one of two perpendicular co-ordinates. In this way it is possible to move the picture-magnification (by means of microscope and distance) of 15000 times enlargement over the small opening of a photocell. The readings are made directly by observing in a microscope the deviations of a filament-electrometer connected with the photocell. Very small areas – especially dependent on the opening and other properties of the photocell and on the magnification – in different structures in one cell can be defined and quantitatively determined by measurements in different focus.

Hammarsten went on to note that in some instances Caspersson employed two photocells, radio equipment, two galvanometers, and a double thermocell.

Caspersson's early application of these instruments contributed significantly to the understanding of the role of nucleic acids in the cell. He determined that under ultraviolet illumination, nucleic acids and proteins had different absorption spectra so that he could measure local quantities of both (Caspersson, 1936). One of his first findings was that it is in cell division that the amount of nucleic acid reaches its maximum. It is important to note that Caspersson did not conclude that the DNA represents genetic material. He rather indicated that the result "points with some probability towards a connection between the duplication of the genes and the presence of nucleic acid" (Caspersson, 1950, p. 96). For him, the genes were proteins. In his collaboration with Jack Schultz, he linked disturbances in nucleic acid metabolism with disturbances in reproduction (Caspersson & Schultz, 1940), but he continued to see the nucleic acids as serving only an ancillary role in the mechanism of self-replication of protein. He commented, "nucleic acids are necessary prerequisites for the reproduction of genes and . . . are probably necessary for the multiplication of self-reproducing protein molecules in general" (Caspersson, 1950, p. 98; summarizing Caspersson & Schultz, 1938).

Caspersson's main approach was to establish correlations involving the nucleic acids and protein synthesis. Some of this work focused on what he took to be the self-replication of proteins in chromosomes during metaphase, which he linked with DNA in the euchromatic region of the chromosome. He traced cytoplasmic proteins to the heterochromatic regions of the chromosome and its apparent relation to the nucleolus during the interphase stage. He interpreted the nucleolus as regulating protein synthesis that occurs in the nucleus just outside the nucleolus. In his report in September 1940, he summarized his conclusions:

> Polynucleotides are a base for the protein synthesis in the cell. A central function for the cell nucleus is to be the centre for the protein production. The

> heterochromatin is an organon regulating the production of the proteins of the cytoplasm. This regulation works via the nucleolus.[13]

In the course of this research, Caspersson differentiated the roles of DNA (ribodesose nucleotides), which he took to be necessary for the synthesis of proteins in the genetic material, and RNA (ribose nucleotides), which figured in the synthesis of cytoplasmic proteins. He found that the "*rapidity* of the protein synthesis in the living bacteria is a simple, almost linear function of the amount of ribose nucleotides." He concluded that this "forms one of the best proofs, not only for the interplay of nucleotides and proteins at the protein synthesis but also for the general validity of this mechanism."[14] Caspersson identified such increases in RNA both in the interphase chromosome, in the nucleolus, and in the cytoplasm. Although drawing a correlation between RNA presence in these locations and protein synthesis, he remained vague about the role played by RNA and continued to construe self-reproducing proteins as the genetic material. He also localized protein synthesis in the nucleus and offered no account of the role of the RNA found in the cytoplasm at the same time as he took the newly made proteins to be diffusing into the cytoplasm. In the course of this investigation, Caspersson also investigated viruses and concluded that they took over the normal protein synthesis mechanisms of the host cells.[15]

In addition to his own research, Caspersson began working during the war with several younger researchers, including Holger Hydén, Bo Thorell,

[13] Letter to H. M. Miller, 1 September 1940, Folder 8, Box 1, Series 800, RG 1.1, Rockefeller Foundation Archives, RAC.

[14] Letter to Frank Blair Hanson on 8 August 1944, p. 2, Folder 9, Box 2, Series 800D, RG 1.1, Rockefeller Foundation Archives, RAC.

[15] Caspersson performed most of this research in the period 1938–45. After that, he focused even more on instrument development and offered little in the way of new findings. Prior to renewing his funding in 1956, the Rockefeller Foundation conducted a review of his work, soliciting evaluations from Francis Schmitt, Alfred Mirsky, H. Stanley Bennett, and Barry Commoner. Warren Weaver expressed surprise at the negativity of these assessments and provided Caspersson one last grant at a much smaller amount than Caspersson had requested. Mirsky's evaluation is instructive: "In 1943 it seemed as if Caspersson's work was highly imaginative and also precise. As time went by it became apparent that Caspersson's claims for accuracy were on the whole spurious. . . . About all that has remained of this work is the suggestion that ribonucleic acids are in some way correlated with protein synthesis. This fruitful idea was advanced independently by Brachet, and it is worth noting that Brachet's observations were made by a simple, qualitative staining procedure – quite a contrast to the imposing instrumentation of Caspersson. For the past fifteen years Caspersson has produced little, if anything of significance" (letter to Gerald R. Pomerat, December 4, 1956; Folder Karolinska Institutet Cell Research, 1956–7, Series 800D, RG, Rockefeller Foundation Archives, RAC). While also noting the dearth of recent biological advances by Caspersson, Bennett commented that Caspersson's institute had trained several important junior scientists.

and Arne Enström. He also regularly hosted a number of visiting scientists. With the move into his new laboratory in 1946–7, Caspersson expanded the range of research techniques employed, adding both electron microscopy and cell fractionation. Of particular note is that electron microscopist Fernández-Morán, whose research I will discuss in the next chapter, worked with the Caspersson group for a couple of years on nerve fiber ultrastructure. Despite this, and despite his becoming the first editor of a new journal, *Experimental Cell Research*, Caspersson did not set the agenda for cell biology. Like Brachet, the focus of his research was limited to nucleic acids, not the full range of cell activity. A major reason for his limited impact may have been his preoccupation with instrumentation and failure to apply the instruments in developing new biological ideas after the early 1940s.[16]

One interesting feature of the pursuits of Bensley, Brachet, and Caspersson is that in all three cases the research projects emerged from existing recognized problems in subdomains of what would become cell biology. Perhaps as a consequence, none of them created a laboratory with the breadth of research in cell biology as emerged at the Rockefeller Institute in the 1940s. Caspersson came the closest by incorporating instruments for both cell fractionation and electron microscopy. However, these techniques played a rather peripheral role as the laboratory's focus remained on cytochemistry.

5. ADDING A BIOCHEMICAL PERSPECTIVE TO THE ROCKEFELLER LABORATORY

Around the time that Claude's focus began to change from cancer to normal cells, the group working with him in Murphy's laboratory expanded. The first to join the laboratory, in 1939, was Keith Porter (I will return to Porter in Section 6). Then, in 1941, George Hogeboom, who had completed his medical degree at Johns Hopkins in 1939, brought skills in cytochemistry and biochemistry. Initially he worked most closely with Murphy on his cancer projects. For example, in his first year, he carried out chemotherapy studies on tetra-methyl-ortho-phenylene-daimine (OTM) and rotenone, substances

[16] An approach very similar to Caspersson's, but more fruitful, was pursued at Columbia University under the leadership of Arthur Pollister and Franz Schrader. Hewson Swift, a graduate student at Columbia during this period, did pioneering research establishing the constancy of the relation between number of chromosomes and amount of DNA measured spectrographically (Swift, 1953). After finishing his degree, Swift moved to the University of Chicago and set up a similar laboratory focused on measuring DNA content of nuclei. He subsequently employed electron microscopy and created an important center for cell biology. He served as program chair for the first meeting of the American Society of Cell Biology and became its third president.

known to inhibit oxygen consumption in lymphosarcoma. He also carried out studies of the comparative permeability and respiration of normal and malignant cells afflicted with lymphatic leukemia. In some cases, though, Hogeboom's work on cancer became a tool for understanding features of normal metabolism. For example, tyrosine oxidase had been shown to catalyze the formation of melanin in plants and insects, but not in humans. In collaboration with Mark H. Adams, Hogeboom used cell fractionation techniques to isolate two enzymes from mouse melanoma cells. They first obtained a supernatant that catalyzed reactions of both tyrosine and dihydroxyphenylalanine (dopa). They then created two different precipitates with different saturations of ammonium sulfate, one of which catalyzed the tyrosine reaction while the other catalyzed the dopa reaction. In addition to identifying some of the characteristics of the two enzymes, they also observed that when centrifugation continued for several hours, most of the tyrosinase activity appeared in the sediment. They concluded, "This finding suggests that the enzyme may be associated with a particulate component of the cell (microsomes, Claude) and offers an explanation of its insolubility after precipitation by ammonium sulfate" (*Annual Report*, 1942–3, p. 85, see also Hogeboom & Adams, 1942).

In the same period Hogeboom was carrying out this work, Claude himself was becoming increasingly aware of the need to collaborate with biochemists in order to ascertain the enzyme constitution of his fractions. In the *Annual Report* for 1940–1, he noted, "Reports from other laboratories have demonstrated the association of these cell components with cytochrome oxidase, succinic acid dehydrogenase and phosphatase activity. Development of methods to extend the study of function is in progress" (p. 74). The following year he reported on a study with Dean Burk from Cornell which showed that both the large and small granule fractions were capable of taking up oxygen and concluded,

> This fact and the presence of relatively large amounts of iron and copper may indicate that these bodies are associated with the oxydoreduction activity of the cell. The larger granules isolated undoubtedly are what have been referred to as "secretory granules." Attempts are now being made to ascertain if special functional activities of various cells are centered in the granules. (Annual Report 1941–2, p. 74)

In 1942–3, Claude began to collaborate with Rollin Hotchkiss, a biochemist who had been in Dubos' laboratory until Dubos left for Harvard in July, 1942. Claude and Hotchkiss focused on d-amino acid oxidase, showing that it was

Table 5.1. ***Results of Enzyme Studies by Claude Hogeboom, Hotchkiss, & Hoagland from the Annual Report for 1943–1944***

Type of Enzyme	Substrate	Supernate	Large Granule	Microsomes
Nuclease + Phosphatase	Nucleic Acid	0	+++	+++ [1]
Ribonuclease	Nucleic Acid	0	+++	+
Phosphatase	Nucleic Acid	0	+++	Trace
Phosphatase	Phosphate esters and ATP	0	+	Trace
Nucleopyridinase	Coenzyme I	0	+	+++
Cytochrome Oxidase	Ascorbic Acid	0	+++	Trace
Succinoxidase	Succinic Acid	0	+++	Trace
Dehydrogenase	α-glycerophosphate	0	+++	Trace
Oxidase	d-amino Acids	0	+++	0
Phosphate Transfer	ATP to Phosphate esters	+++	Trace	0
Catalase	Peroxide	+++	+	Trace
Malic Dehydrogenase	Malic Acid	+++	Trace	Trace
Coenzyme I	(as growth factor)	+	++	+

found only in what they referred to as the secretory or large granule fraction. In the *Annual Report* for that year, Claude indicated an intention to extend the investigation to other enzymes. These studies, involving Claude, Hogeboom, Hotchkiss, and Charles L. Hoagland, began in earnest in 1943–4 and Table 5.1 shows their results (as stated in the *Annual Report* for that year).

During the following year, these studies, except for investigations into ribonuclease, were suspended as Hogeboom was diverted to war-related research. When Hogeboom finally returned in September 1945 there was a critical change in the way the investigators pursued this research. In addition to simply indicating how much activity a particular fraction exhibited, the researchers compared the amount of activity quantitatively with the amount exhibited by the initial extract (the supernatant from which tissue debris, free nuclei, and red blood corpuscles had been removed in the initial centrifugation). This was done using the Warburg manometer to supply a substrate (e.g., succinic acid salt) to the fraction and determine the resulting rate of oxygen gas uptake. This rate was taken as a measure of the extent to which the relevant enzyme or enzyme system was present and active in the fraction. For example, succinic acid salt provided a measure for succinic oxidase (the enzyme

system that includes succinic dehydrogenase plus enzymes in the cytochrome chain). Similarly, cytochrome *c* was supplied to the same fraction to obtain a measure for cytochrome oxidase. They then divided the amount of activity in a fraction with that from the initial extract. They reported that over three experiments on average 70% of the cytochrome oxidase activity and 74% of the succinoxidase activity was found in the large granule fraction, while less than 4% of the cytochrome oxidase activity and 7% of the succinoxidase activity was found in the microsome fraction (Hogeboom et al., 1946). They attributed the bit of activity in the microsome fraction to large granules or large granule fragments that corrupted it, and claimed that dark-field microscopic examination of the microsome fraction indicated the presence of a sufficient number of large granules to support this explanation. They traced most of the remaining activity of these enzymes to large granules in sediments that were discarded in the process of purifying the different fractions.

Whereas prior to the war the team was satisfied to determine what amount of the activity associated with an enzyme could be found in a given fraction, they now sought to link the activity of a given enzyme with only one fraction. This was the beginning of the one enzyme–one fraction approach to interpreting cell fractionation results discussed in Chapter 4. Adopting this approach, they claimed that "Taken together, these observations suggest that the cytochrome oxidase and succinoxidase systems . . . are entirely localized in the so called large granules" (Hogeboom et al., 1946, p. 626).

Subsequent to this research, two additional investigators joined the laboratory, Walter Schneider and George Palade. Schneider had completed his Ph.D. at one of the top biochemistry departments in the U.S., the University of Wisconsin, in 1945. Palade, a native of Romania, had studied medicine at the University of Bucharest, where he carried out physiological research on the kidneys of dolphins. During the war, he taught at the Department of Anatomy at the University of Bucharest, then emigrated in 1946 to New York City. There he first worked with Robert Chambers at New York University on cellular membranes but, upon seeing Claude's first micrographs (discussed below), started to volunteer in Claude's laboratory.

Together Hogeboom, Schneider, and Palade continued the investigations into the biochemistry of cell fractions.[17] A serious shortcoming of the earlier

[17] While his colleagues were refining the techniques for identifying enzymes with particular cell fractions, Claude pursued the question of whether the large granule fraction could itself be differentially fractionated. This would soon become a major endeavor of biochemists (see Chapter 6), but as early as Claude (1946), he reported separating a small particle component from mitochondria containing most of the ribose nucleic acid associated with large granules. He claimed these small particles from within mitochondria could be identified with elements approximately

studies was that it was still not possible to conclude with certainty that Claude's large particles constituted mitochondria. A major reason was that when examined microscopically, the particles in the fraction did not exhibit the rod-shaped appearance of mitochondria and did not respond to the usual mitochondrial stains. One of the main strategies of the new team of researchers was to vary the media used for cell fractionation. Although Claude had tried several variations in fractionation techniques, he had continued to use either distilled water or saline solutions as the media. Hogeboom, Schneider, and Palade (1948) explored sucrose as well as other sugar solutions. With approximately isotonic (0.25 *M*) sucrose, they noted that the large granules did not agglutinate and were roughly of the same size as with isotonic saline. The particles, however, did not retain the elongated shape of mitochondria. When they tried more hypertonic sucrose solutions, the particles became more elongated, with the percentage of rod-like shapes reaching a maximum with 0.8 to 1.0 *M* sucrose solutions. The researchers therefore adopted 0.88 *M* sucrose solutions for their research.

Not only did the large granules now more closely resemble mitochondria as observed in whole cells, they also stained with Janus green B, a stain selective for mitochondria. The researchers also dispelled Claude's claim that the large granule fraction was partially or even largely comprised of secretory granules. Secretory granules stain with neutral red and once mitochondria were made to retain their rod-like shape, the two could be distinguished. The secretory granules appeared to break up after the rupture of the cell membrane and to migrate centripetally; they were thus not part of the large granule fraction. Having developed a fraction that was demonstrably mitochondrial, the researchers replicated the findings of the earlier study that this fraction housed the succinoxidase system. They now said, more boldly than in 1946, "The mitochondrion can therefore be considered as a complicated functional unit possessing two of the most important respiratory enzyme systems of the cell . . ." (Hogeboom et al., 1948, p. 360).

With the improved fractionation procedures, Hogeboom and his colleagues continued the quantitative analysis of the composition of the different fractions. In addition to confirming that the mitochondria contained nearly all the succinoxidase, they established that the microsome fraction contained about 50% of the pentose nucleic acid. From his earliest studies of the small particle fraction, Claude had noted these particles were high in ribose nucleic acid (RNA). That, however, seemed to be the only clue to their function, and so

0.1 μ in diameter that he and Fullam had identified in mitochondria in their electron microscope studies (see below).

Claude as late as his Harvey Lecture (1948) generally pleaded agnosticism about their function:

> Because of their abundance and their universal distribution, it is reasonable to assume that microsomes play a fundamental role in the economy of the cell. . . . [S]ince the microsomes were isolated in the laboratory, some ten years ago, no sure clue has been found to reveal their function although several attempts have been made, on theoretical grounds, and especially because of their high content in nucleic acid, to have them play some important role in the cells, either as plasmagenes, or agents of protein synthesis. (p. 142)

Claude doubted the findings of both Caspersson and Brachet regarding the role of RNA in protein synthesis, because he questioned how protein synthesis could proceed once separated from the site of oxidative respiration. Instead he suggested that RNA may be involved in anaerobic respiration "either in some phase of the anaerobic mechanism, or act as intermediate in the energy transfer for various synthetic reactions" (p. 163). The basis for this speculation was the correlation of both RNA and fermentation in yeast and some bacteria.[18]

While agnostic about the function of the microsomes, Claude was far from agnostic about the function of the mitochondrion. The localization of key oxidative enzymes in the mitochondrial fraction led him to conclude that it was the locus of the key oxidative processes that provide the bulk of the energy for cell functioning. As he put it in his Harvey lecture, "mitochondria may possibly be considered as the real power plants of the cell" (Claude, 1948, p. 137).

6. ADDING ELECTRON MICROSCOPY AS A TOOL

As discussed in Chapter 4, in 1943–4 Claude took advantage of an opportunity to use the electron microscope at Interchemical Corporation to examine the

[18] "From these considerations one might venture the conclusions, partly facts and partly hypothesis, that, whereas most of the metabolic activity of the cell is found in the cytoplasm, the supply of energy may be segregated in various cytoplasmic entities: the aerobic respiration in the mitochondria, as already demonstrated, the anaerobic processes in the ground substance. This might explain the intense basophilia of cells in young embryos, in fast growing tissues, and in tumors, especially in areas where the circulation and the fresh supply of oxygen may be inadequate or defective. Demonstration that a relation exists between the power of anaerobic glycolysis and ribose nucleic acid distribution would permit us to consider further the possibility that the nucleus, where the cytochrome-linked respiratory system is apparently lacking, derives its energy at least in part, from loci where ribose nucleic acid is present, especially the nucleolus, and certain chromosomal regions" (Claude, 1948, p. 163).

large and small granules generated by his fractionation procedure. Claude described his first observations in the *Annual Report* for that year:

> The large particles from liver and mitochondria from leukemic cells under the electron microscope appear as perfect opaque spheres 0.5 to 1.6 μ in diameter. No definite membrane is evident in the prints. In instances where the granules are injured and some loss of substance has occurred, internal structure may be seen. Among these are small particles in the size range of microsomes. This observation taken with the fact that in the test tube disrupted large granules yield what appear to be microsomes suggests a relationship between these formations. (pp. 69–70)

He was not able to see any structure in the undamaged mitochondria because of their thickness, but if they had damage that allowed some material to escape, or were flattened somewhat during mounting, then the electron beam could penetrate them. Although Claude admitted in his published report (Claude & Fullam, 1945) that he could not see a membrane in the micrographs, he inferred its presence from the behavior of mitochondria in which some contents had escaped: "In some cases, loss of substance seems to release the tension which keeps the mitochondrial body spherical and, in such circumstances, the impression is gained that mitochondria possess a differentiated covering which may become more or less completely separated from the mitochondrial mass" (p. 57). Claude observed that mitochondria retained their shape and remained discrete if he used sufficient salt in the media employed in fractionation to insure proper tonicity. In opposition to Bensley's position, he contended that this disproved "the assumption that mitochondria may be formed by the concretion of substances preexisting in the cytoplasm or that they may disappear and reappear in living cells because of changes of equilibrium occurring in the surrounding protoplasm" (p. 58).

Claude's first use of the electron microscope provided little information about either mitochondria or the microsomes, but another approach would prove much more fruitful. The key person in this development was Keith Porter, who had joined the laboratory in 1939. Porter had completed his doctorate a year earlier at Harvard, pursuing research on the development of frog embryos with only a haploid set of chromosomes. This research required him to develop skills in micromanipulation of cells (e.g., removal of the nucleus before insemination so that the egg would develop with only the sperm's chromosomes). After he received his Ph.D., Porter was a postdoctoral fellow at Princeton, where he began to transplant the haploid nucleus of a frog of one geographically isolated race or subspecies into enucleated frog embryos of different races (Porter, 1941a; Porter, 1941b). Porter's ability to carry out these

transplants suggested to Murphy that Porter might be capable of transplanting the hypothesized tumor particles from an infected cell to other cells. When he came into the laboratory, though, Porter continued the transplant studies he had been pursuing. His goal was to demonstrate cytoplasmic influences upon development by comparing embryos into whose cytoplasm he transplanted the nucleus of a different race with normal embryos of that race. The results indicated a genetic effect of the cytoplasm as well as of the nucleus. Given the focus of the laboratory on cancer, though, Porter also began to investigate the inhibition of growth from carcinogenic agents, including X-rays and such chemicals as methylcholanthrene. He examined both the effects of different dosages on tail regeneration in the newt and the character of the tissue reaction induced.

For a number of years, Porter's research seemed to be largely independent of other investigators in the laboratory. However, as Claude was exploring the use of the electron microscope, the value of having Porter in the laboratory was realized when Porter proposed using tissue-cultured cells to produce sufficiently thin specimens for electron microscopy (see Chapter 4). Working with Claude and Fullam at Interchemical Corporation, Porter produced a composite electron micrograph (from several pieces imaged separately) of a whole chick embryo cell. Figure 5.1 shows this micrograph and a comparable one from a light microscope that they included for comparison. The nucleus was generally too thick to observe anything but the nucleoli, which appeared as less dense than the rest of the nucleus. Parts of the cytoplasm, though, generated a detailed image. The following is the figure caption in which Porter et al. presented their interpretation:

> Electron micrograph of a fibroblast-like cell, and nerve fibers cultured from a chick embryo tissue. Differential absorption and scattering of electrons by the cytoplasmic area has silhouetted a number of structural details among which are: filamentous mitochondria of various lengths and fairly constant width; scattered, small elements of high density especially abundant around the nucleus and presumably representing Golgi bodies; and a delicate lace-work extending throughout the cytoplasm. The nucleus is visible but multiple scattering of electrons due to excessive thickness results in considerable blurring. Three nerve fibers can be seen: one crossing the upper part of the picture and having no connection with the cell; one ending in contact with the cell surface at the right; and one at the lower part of the picture also in contact with the cell surface. This latter has the appearance of a growth cone. Details of the cell's margin and extensions are clearly defined. The arrows point to extensions mentioned in the text as "jagged points" (a) and "finger-like processes". (1945, p. 246)

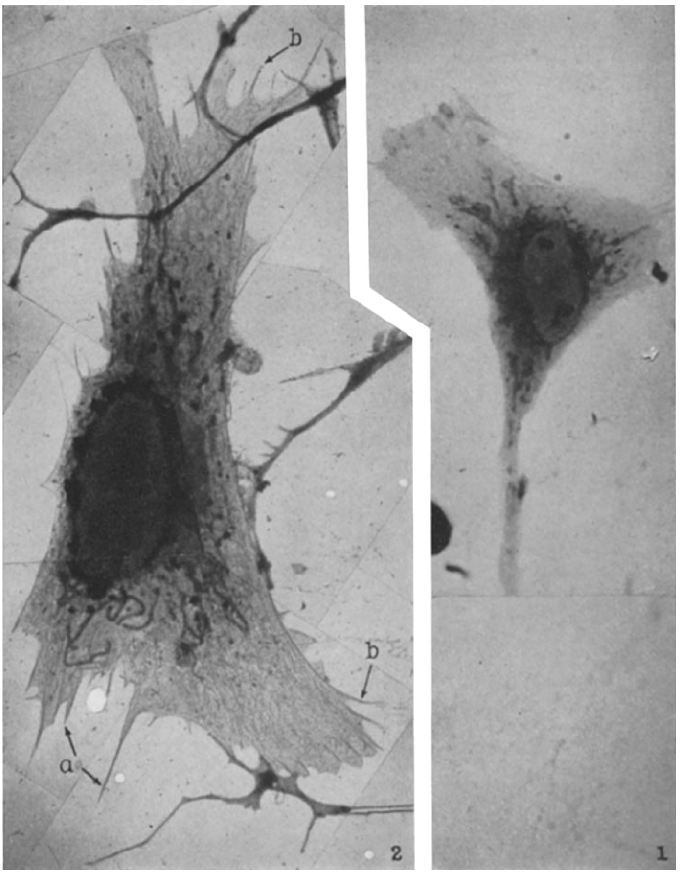

Figure 5.1. On the left is Porter, Claude, and Fullam's original 1945 electron micrograph of a fibroblast-like cell and nerve fibers cultured from chick embryo tissue. On the right is a photomicrograph of a similar cell using a light microscope. Reproduced from Porter, K. R., Claude, A., & Fullam, E. F. (1945), A study of tissue culture cells by electron microscopy, *Journal of Experimental Medicine, 81*, pp. 235–255, Figures 1 and 2 on plate 10, by copyright permission of The Rockefeller University Press.

As they noted, the mitochondria appeared as elongated rod-like structures that appeared to have areas of increased density as well as extremely small granules 10 to 20 m μ in diameter. They proposed that "these may be composed mainly of inorganic salts, or that they represent centers where reduced osmium has accumulated" (239). Due to the osmophilic character, they interpreted the dense bodies with angular outlines in the micrographs as Golgi bodies. In the *Annual Report* for 1944–5 they appealed to these observations to challenge the interpretation of the Golgi apparatus as an artifact of the way

the bodies cluster around the nucleus, noting that "the resolving power of the light microscope would not separate them and as a consequence they would appear as a complex" (p. 72). (Recall from Chapter 4, though, that five years later Claude would join with Palade to argue that the Golgi was indeed an artifact.)

Porter (1955–6, p. 175) characterized the goal in making these early micrographs as "to see what there might be in the optically empty parts of protoplasm."[19] Over the years several authors had proposed the existence of a cytoskeleton that was responsible for maintaining the structure of the cell (Needham, 1942; see also Bonner, 1952; Peters, 1930; Picken, 1940). With respect to this claim, the micrographs produced novel and controversial results. In the *Annual Report* Porter and Claude referred to the ground substance as *spongioplasm* and reported that in the micrographs it appeared to be comprised of particulate elements 30 to 150 m μ in diameter. In the published paper they commented on the status of these structures:

> It is not known whether the particulate elements just mentioned pre-exist in the living protoplasm, or whether they are artifacts arising from the cell body or the cell wall, as a result of fixation or drying. In this connection, however, it is of interest to recall that experiments in this laboratory have shown that the chromophilic ground substance is sedimentable and, therefore, probably particulate in nature. Touching on this problem also is the fact that small particles, or microsomes, estimated to average about 70 m μ in diameter have been previously isolated from extracts of normal chick embryos and Chicken Tumor I. (Porter et al., 1945, p. 238)

The authors then proceeded to a second observation – a lace-like reticulum running through the thinner parts of the cell.[20] They reported that "vesicle-like

[19] Porter (Interview, 1987, University of Maryland, Baltimore County) recalled that he had no particular hypothesis in mind in generating the first micrographs, although he was convinced from dark-field light microscopy that there had to be more structure than light microscopy could reveal. Rather, the availability of tissue-cultured cells provided "an excuse" to see what the electron microscope might reveal. The experience of seeing the first micrographs apparently created a "flash-bulb memory" for Porter. He related to me with obvious excitement, "I remember the night so distinctly I could play it back. It was in the war. New York City was blacked out. It was cold and raining and black, was it black. And we were trying to find the entrance to the building on 48th Street, West Side. We got this thing, I think there was only one cell on the grid, but it was between the bars and we took picture after picture of it, we must have taken 30 or 40 micrographs of it so that we could piece together the whole thing. And I was fascinated, I was really fascinated. I don't think I slept at all that night. We didn't leave there until 3:00 A.M."

[20] Porter (Interview, 1987, University of Maryland, Baltimore County) related that initially they had "no idea what the lace-like stuff was inside the cytoplasm." Of particular interest was the fact that it fragmented in tissue-cultured cells, which suggested that while it was incapable

bodies, i.e., elements presenting a center of less density, and ranging in size from 100 to 150 m μ, can be seen arranged along the strands of the reticulum just mentioned" (p. 238).

Electron microscopy gave Claude, again in collaboration with Porter, an opportunity to return to cancer research by examining tumor cells. Already in the *Annual Report* for 1944–5, they described finding in tumor cells "small granules of uniform size and characterized by a density appreciably greater than that of the surrounding cytoplasm . . . which are certainly not common in normal cells" (p. 73). The next year they conducted studies on Chicken Tumor 1 and Chicken Tumor 10 cells, identifying granules of similar structure that "are readily distinguished from normal cell constituents, especially from microsomes, by their regular granular shape, their relatively uniform size and because of a greater density in osmium preparations" (p. 91). The particles were differently distributed in the two tumor types, with those in Chicken Tumor 10 being clustered in colonies while those of Chicken Tumor 1 were distributed more widely in pairs or in rows of up to six particles. By the time of their published paper in 1947 they were willing to conclude that these particles were the causative agent of the respective tumors (Claude et al., 1947). At the end of the paper they speculated that the particles replicate by division and noted that the variation in size of the particles was compatible with their growing larger prior to such splitting and yielding smaller particles after the split.

After their joint investigation of cancer cells, Porter and Claude went in their own directions. Claude directed his attention to developing a technique for thinly sectioning cells while Porter continued to develop the technique for studying cultured cells and understanding the "lace-like reticulum" found in them. Already in the *Annual Report* for 1945–6 he had become more definite about the relation between granules and the lace-like reticulum of the endoplasm:

> Possibly the most important discovery from these studies is that in relation to the endoplasm. This cell system has been heretofore unobservable & only dark field illumination has provided any hint of its existence. As seen in osmium-fixed preparations under the electron microscope, it appears as a finely divided reticulum along the strands of which are scattered tiny bodies 60 to 100 m μ in diameter. The particulate components are believed to be the same as those isolated by high-speed centrifugation and known as microsomes. This endoplasm

of stretching as much as required in thin-spreading tissue-cultured cells, it was embedded in something that did stretch. It was not just suspended in the cytoplasm, because otherwise it would not be stretched but, rather, would remain in the middle.

> appears from recent observations to be derived from the activities of small dense unnamed granules which in a few normal cells can be seen in what appears to be the act of division. (p. 88)

The following year (1946–7) Porter said of the endoplasm that

> the evidence is convincing that it is the most active part of the living unit. It is present in one form or another in every cell so far examined. It makes up a fibrillar apparatus concentrated around the central body and sends fine extensions of its substance throughout the cell. The vesicular structure of the small units suggests a secretory activity. In as much as it is the only cytoplasmic system showing some structure and continuity, it may be assumed that it determines the mosaic character of the egg cell. (p. 80)

Porter also engaged in his own studies of cancer cells. In his first efforts he, together with visiting fellow Helen Thompson, examined cultured cells from three different rat sarcomas (the *Annual Report* for 1946–7 indicates four sarcomas). These cells, they claimed, exhibited a much greater density of endoplasmic granules located on shorter strands (Porter & Thompson, 1947). A second study involved mammary carcinoma in mice. John Bittner (1936) had discovered that this cancer was transmitted through their mother's milk. With Thompson, Porter used the electron microscope to examine mammary gland tumor cells grown in tissue culture from mice. They identified within them distinctive particles about 130 m μ in diameter with a dark, well-defined central core. Although the evidence was only circumstantial, they proposed "tentatively" that the particles were the viral agent in the milk (Porter & Thompson, 1948).

7. THE STATE OF CELL STUDIES AT THE END OF THE 1940s

As I will discuss at the beginning of the next chapter, in 1949 and 1950 the research laboratory at Rockefeller underwent a major transformation and the research efforts broadened to other laboratories. So we reach a natural transition and it is worth drawing together just what was accomplished during the 1940s in that laboratory (see Claude, 1950, for a useful recapitulation). The decade began with Claude's determination that normal cells contained particles of the same size as he had isolated in his tumor-causing fraction from Rous sarcoma cells. His research increasingly focused on the constitution of normal cells (although he would return to the attempt to identify cancer particles later in the decade). Initially cell fractionation was his primary tool, and with it, he developed standardized procedures to segregate

two fractions of cytoplasmic particles plus a nuclear fraction and a supernatant. Eventually Claude revised his initial assessment of the two fractions, accepting that the larger particles were primarily comprised of mitochondria while the small particles were a new cell component he labeled *microsomes*. In the second half of the decade, he and his collaborators refined the procedures for fractionation, especially the choice of media, and began to associate particular enzymes with specific fractions. Claude also introduced another new technique, electron microscopy, initially to examine the isolated fractions and then, in collaboration with Porter, to examine whole cells grown in tissue culture. The latter approach permitted identifying both mitochondria and a lace-like reticulum. They found that the reticulum was related to the microsomes isolated by cell fractionation.

The view of cytoplasm developed through this research was that it was comprised of two primary types of structures – mitochondria and the lace-like reticulum/microsomes – plus a gel-like aqueous component – the "cell sap" or cytosol – corresponding to the supernatant. The two structures were associated with different cell activities. Both morphologists and biochemists rapidly accepted Claude's characterization of the mitochondrion as the power plant of the cell (as we will see in the next chapter). Although Claude remained agnostic about the function of microsomes and related structures in the endoplasm, others such as Brachet and Caspersson had concluded that they figured in protein synthesis. This differentiation of function provided the foundation for a functional decomposition of the cell into organelles in which were situated mechanisms that contributed differentially to cell life. The further development of this account of cellular mechanisms required two additional steps: (1) decomposing the already discovered organelles to show how their component parts contributed differentially to their functioning, and (2) finding the organelles presumed to be associated with other cell functions. A research community that rapidly increased in size energetically pursued these goals in the 1950s and 1960s.